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In the Application of:	Richard L. Edelson	)	
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		)	Art Unit: 1632
		)	
		)	Examiner: Quang Nguyen
		)	
	METHODS FOR INDUCING THE DIFFERENTIATION OF MONOCYTES INTO FUNCTIONAL DENDRITIC CELLS AND IMMUNOTHERAPEUTIC COMPOSITIONS INCLUDING SUCH DENDRITIC CELLS	)	
		)	
Serial No.:	09/294,494	)	
Filed On:	April 20, 1999	)	(Docket No. 270425.0003)

Dated at Hartford, Connecticut this 14th day of August, 2001

ATTN: BOX RCE
Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. RICHARD L. EDELSON

STATE OF CONNECTICUT)
) ss.
COUNTY OF NEW HAVEN)

I, Dr. Richard L. Edelson, do hereby declare and say as follows:

I received a Bachelor of Arts degree from Hamilton College in 1966 and a Medical Doctorate from Yale University School of Medicine in 1970. I performed my internship at the University of Chicago, my residency at Massachusetts General Hospital, and I was a senior resident at Columbia Presbyterian Hospital.

I am currently Chairman of the Department of Dermatology and Deputy Dean of Clinical Affairs at the Yale University School of Medicine. I am an author or co-author on numerous published articles, including several articles on treatments for cutaneous T cell lymphoma and the use of human dendritic cells for immunotherapeutic treatments.

I am an inventor of the above-referenced patent application regarding compositions comprising functional antigen presenting dendritic cells for use in immunotherapeutic compositions. As described in the application, the composition of the invention comprises dendritic cells having a more uniform age profile than compositions produced by prior methods.

This feature is quite advantageous for two reasons. First, dendritic cells most efficiently phagocytose apoptotic cells during an early period of their life cycle. Hence, having a predominantly immature dendritic cell population increases the uptake and processing of antigens associated with apoptotic disease causing. Accordingly, if a composition contains a population of dendritic cells having a relatively uniform immature maturity, the number of functional antigen presenting dendritic cells available for immunotherapeutic treatment can be maximized.

Second, the uniformity of age of the antigen-loaded dendritic cells is critical for their modulation. For example, immature antigen-loaded dendritic cells are most effective at causing immunologic tolerance to the relevant antigen, while more mature antigen-loaded dendritic cells are most effective at inducing a positive immune response. Therefore, a homogeneous population of antigen-loaded immature dendritic cells would be desirable in the treatment of patients experiencing organ transplant rejection, graft-versus-host disease and autoimmune disorders. A homogeneous

population of antigen-loaded mature dendritic cells is also desirable in the induction of anti-tumor immune responses.

Since the process described in the application initiates conversion of monocytes to dendritic cells in synchronous manner, as opposed to conventional approaches, the process results in a composition comprising dendritic cells of uniform age. Therefore, the dendritic cells in compositions prepared by the process are preferable to those produced in a standard cytokine induced system for inhibition of auto-aggressive immune reactions, as well as induction of anti-tumor immune responses.

To demonstrate that the compositions of the present invention contain an enhanced number of dendritic cells having a similar age as compared to compositions produced by prior art incubation methods, a sample of positive control normal human peripheral blood leukocytes were treated using the novel process of the present invention, and a sample of positive control normal human peripheral blood leukocytes were treated using a standard and widely used method of inducing monocyte differentiation to dendritic cells. The populations of dendritic cells in the samples were then measured and compared using well known cell markers.

A first sample of positive control normal human peripheral blood leukocytes was treated by pumping the sample as a 1mm film between the plastic walls of a device to induce transient adherence by the passaged monocytes to the plastic walls of the device. After the sample was pumped through the plastic device, the sample was incubated for 24 hours. This method of treating the blood sample is referred to below as the "Novel Treatment."

A second sample of positive control normal human peripheral blood leukocytes was treated by exposing the sample to a combination of granulocyte-

monocyte colony stimulating factor (GM-CSF) and Interleukin 4 (IL-4) and incubating the sample for 24 hours. This treatment is referred to in Table I below as "Conventional Treatment."

The relative numbers of monocytes and dendritic cells produced after 24 hours was determined using four commonly used cell markers. The results are summarized in Table I.

TABLE I

Percentage of Cells Exhibiting Marker

Cell Marker	Conventional Treatment	Novel Treatment
CD14	39%	20%
CD36	36%	52%
cCD83	15%	22%
mCD83	4%	15%

As set forth in Table I, by each of several determinations, the Novel Treatment was substantially more efficient than Conventional Treatment in causing rapid conversion of monocytes to immature dendritic cells. It is noteworthy that this monocyte-to-dendritic cell conversion, by the Novel Treatment, was accomplished without the addition of special growth factors (cytokines) required by the less efficient Conventional Treatment.

In a typical determination, after 24 hours of incubation using the Conventional Treatment, 39 percent of the large mononuclear cells in the sample

continued to express the monocyte marker CD14, compared to only 20 percent of the mononuclear cells treated by the Novel Treatment, i.e., by this determination twice as many monocytes had been induced by the Novel Treatment to enter the dendritic cell maturational pathway. This percentage of cells expressing the CD14 marker demonstrates that the Novel Treatment directs a greater percentage of monocytes into the dendritic cell pathway.

Once in the dendritic cell pathway, the transitioning mononuclear cells begin to develop markers characteristic of dendritic cells. Expression of CD36 occurs in monocytes transitioning toward the dendritic cell stage. As summarized in Table I, 36 percent of the monocytes treated using the Conventional Treatment expressed CD36, while 52 percent of the monocytes treated using the Novel Treatment expressed CD36, i.e., 50 percent more cells process by the Novel Treatment had acquired this early marker of progression into the dendritic cell pathway. This finding further demonstrates that the Novel Treatment induces a larger fraction of monocytes to begin to transition to dendritic cells.

The next step along the dendritic cell pathway is the development of cytoplasmic CD83 (cCD83), as a prelude to the display of cell membrane CD83 (mCD83). As summarized in Table I, whereas only 15 percent of the monocytes treated using the Conventional Treatment had developed cCD83, 22 percent of the monocytes treated using the Novel Treatment displayed cCD83. This further demonstrates that the Novel Treatment induces about 50 percent more of the monocytes to enter this early stage of the dendritic cell pathway.

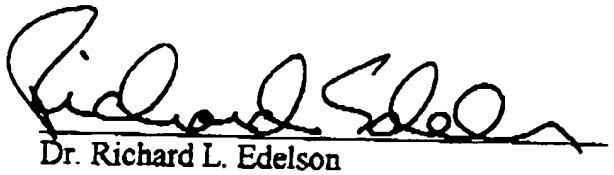
Expression of membrane CD83 (mCD83) depicts the next stage of dendritic cell maturation. It is young dendritic cells at this stage of development that are

particularly adept at internalizing antigenic apoptotic cells and processing and presenting the contained antigens. As summarized in Table I, only 4 percent of the cell population treated using the Conventional Treatment expressed mCD83. In the sample treated using the Novel Treatment, 15 percent expressed mCD83, nearly a four fold increase over that obtained by the conventional method. This clearly demonstrates that the Novel Treatment induces a significantly higher percentage of monocytes to differentiate into precisely those dendritic cells capable of performing the functions necessary to initiate steps required for development of a cellular vaccine.

These results demonstrate that a composition comprising functional dendritic cells produced using the Novel Treatment of the present invention contains a larger percentage of dendritic cells than compositions produced using prior conventional methods within the 24 hour incubation period. Production of a large number of functional dendritic cells in this relatively short time period is particularly advantageous for the processing of tumor, or other cellular antigens, derived from freshly isolated sources of disease causing cells. Longer preparatory times for production of functional immature dendritic cells is markedly disadvantageous, because of the continued decomposition of apoptotic cells, which become progressively less useful sources of cellular antigens with additional time. In short, the increased efficiency, and ease, of the Novel Treatment in preparing the dendritic cell preparations provides major advantages in the preparation of cellular vaccines that could be useful in human immunotherapy.

These results clearly demonstrate that the compositions produced by the Novel Method can be distinguished from compositions produced by the prior methods using conventional cell markers well known to those in the art.

I, the undersigned, declare further that all statements made herein are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Dr. Richard L. Edelson

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